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Crambe abyssinica a non-food crop with potential for the Mediterranean climate: Insights on productive performances and root growth

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ABSTRACT

Within a framework of renewed interest in crambe (*Crambe abyssinica* Hochst ex R.E. Fries) sourcing raw materials for the bio-based industry, the adaptability and productive performances of this oil crop have been evaluated under contrasting Mediterranean environments (i.e., a fertile site in the northern part of Po valley vs. a semi-arid site of southern Sicily) during two consecutive growing seasons, aiming at its possible stable introduction in this area. The trial set in northern Italy compared three commercial varieties of crambe (Galactica, Nebula and Mario) in spring sowing, while in southern Italy only the var. Mario was tested with autumn sowing. Regardless of location and variety, thermal time for maturity was quite stable (1200–1400 °C), and the crop provided satisfactory seed yields (grand mean 2.29 Mg hulled seeds ha^{-1}), with average oil content of ~400 g kg^{-1} (on dehulled seeds) and ~52% of erucic acid. Significantly higher seed and oil yields were reached in northern than in southern Italy. Furthermore, crambe thermal use efficiency (THUE) was also higher in the north than in the south, possibly due to better environmental adaptability of the crop. The limited intraspecific variability within crambe was confirmed, with better productive performances showed by the domestic selection Mario. Promising traits were revealed in Nebula, showing greater seed weight, root length density and area, and thinner roots, although the root growth of crambe was generally modest compared with modern high erucic acid rapeseed hybrids. Available crambe varieties could be efficiently included in crop rotations across a wide range of environments within the Mediterranean basin. The short growth cycle represents an outstanding added value for this species, allowing the avoidance of prolonged drought and heat stress typical of late spring/early summer months under the Mediterranean climate. However, increased yields are needed to meet the market requests; nonetheless, the little genetic variability suggests that there is large scope for future breeding improvements, maybe exploiting advanced techniques to improve the existing genetic resources.

1. Introduction

The most widely cultivated oilcrops mainly contain five fatty acids in their oil, which differs in relative proportions depending on species, i.e., palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and α-linolenic acids (C18:3) (Carlsson et al., 2011). This means that the oleochemical industry is steadily searching for new sources of uncommon fatty acids for specific applications. High-erucic acid (C22:1) oils are potential raw materials for both oleochemical transformation and direct use in producing erucamide – a slip agent enabling manufacture of extreme-temperature resistant plastic films (Walker, 2004). High erucic acid rapeseed (HEAR) (*Brassica napus* L. var. *oleifera* Metzg) has been the most common "green" source of erucic acid so far, in view of its good yield and adaptability to a variety of environments. This has intensified specific breeding programmes, making several winter HEAR varieties available on European markets in recent years, together with the latest composite hybrid hybrids (CHH) (Zanetti et al., 2009a), now cultivated in the continental climates of central Europe. In warmer and semi-arid environments, inter-

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esting and alternative sources of erucic acid are *Brassica carinata* A. Braun, *Brassica juncea* L. Czern and *Crambe abyssinica* Hochst ex R.E. Fries, all characterised by limited frost resistance. *Crambe abyssinica* (crambe) is particularly interesting, as its oil contains greater amounts of erucic acid than HEAR (≥55% vs. ~50%) (Bondioli et al., 1998; Lazzeri et al., 1994; Temple-Heald, 2004). Crambe was first studied a few decades ago (Laghetti et al., 1995; Lazzeri et al., 1994, 1995; Lessman, 1990; Meijer et al., 1999; Vollmann and Ruckenbauer, 1993; Zanetti et al., 2006), but its cultivation in Europe still remains limited (Fontana et al., 1998; Zanetti et al., 2013). Currently, there is a renewed interest toward this species, as demonstrated by several European projects recently funded within the Seventh Framework Programme (FP7) (e.g., ICON, EPOBIO) and Horizon 2020 (e.g., COSMOS). Some of these projects aimed in particular at improving the oil profile, to achieve further increases in erucic acid contents, with a target rate exceeding 66%, partly with GM breeding techniques (Chhikara et al., 2012; Li et al., 2012; Napier et al., 2014).

Native to the Mediterranean basin (Leppik and White, 1975), crambe has favourable agronomic traits such as short growing cycle (Golz, 1993), tolerance to drought, good adaptability to poor sandy soils, and natural resistance to insects (Anderson et al., 1992; Kmec et al., 1998). In addition, it has indehiscent pods, which prevent shattering and seed loss, unlike HEAR which may be subjected to significant yield losses, especially in the Mediterranean environment, as a consequence of sudden decreases of the atmospheric humidity at maturity.

Despite these favourable traits, crambe is not able to tolerate low temperatures (Leppik and White, 1975; Papathanasiou et al., 1966; Wang et al., 2000) nor water logging, so that only spring sowing can be applied in continental European regions with winter temperatures falling to −7 °C. In southern Mediterranean regions crambe adapts well to late autumn sowing (i.e., late November, early December), like winter wheat, but in view of its shorter growing cycle, harvesting usually takes place more than one month prior to winter cereals, thus avoiding the occurrence of prolonged drought and heat stress typical of late spring/early summer months. In view of the possible stable introduction of crambe in the Mediterranean basin, and considering the limited number of studies conducted in this area (Fontana et al., 1998; Laghetti et al., 1995; Lazzeri et al., 1995), a two-year field trial was carried out in two contrasting environments: at Legnaro (Padova, North Italy), and at Pozzallo (Ragusa, South Italy), with the aim of investigating productive potential of this species and at identifying possible significant constraints. In both locations, phenological stages and productive traits were recorded together with climatic variables in order to estimate the thermal use efficiency of a common variety grown in the two environments. In addition, a full characterisation of the root system of three commercial European varieties was carried out in northern Italy together with their productive and qualitative performances.

2. Materials and methods

2.1. Tested varieties

Three commercial varieties of crambe, Nebula and Galactica, both selected by DLO – Wageningen (NL), and Mario, patented by the Agricultural Research and Economic Study Council – CREA (Bologna, I), were cultivated in open fields for two years in NE Italy, whilst in the southern Italy location only Mario was tested for two subsequent growing seasons. The two "*Dutch*" varieties were selected for their adaptability to the central European environment and were based on F8 selection starting from a common parent, P2 (Mastebroek and Lange, 1997), crossed with "*American*" P1 for Galactica and P3 for Nebula, respectively. According to the origin of the parents, these varieties can be referred to a "*Dutch* × *American*" background for Galactica, and "*American*" only for Nebula (see Mastebroek and Lange, 1997 for further details). Instead, var. Mario was derived from the accession 'BelAnn', unlike P2 and P3, and improved for Mediterranean environmental conditions (Lazzeri L., pers. comm.).

Table 1
Main physical and chemical characteristics of the soils in two experimental sites in the north (Legnaro, PD) and south (Pozzallo, RG) of Italy.

Parameter		Northern Italy	Sourthern Italy
Sand[a]	%	28.6	37.0
Loam[a]	%	56.6	25.0
Clay[a]	%	14.8	38.0
pH (in water)		8.4	8.5
Organic matter[b]	%	2.5	2.6
Total N[c]	$mg\,g^{-1}$	0.9	1.6
Available P[d]	$\mu g\,g^{-1}$	35.5	52.3
Available K[e]	$\mu g\,g^{-1}$	117	325.5
Total S[f]	$\mu g\,g^{-1}$	61.0	NA

NA: data not available.
[a] Gattorta (Lotti and Galoppini, 1980).
[b] Walkley and Black (AOAC, 1990).
[c] Kjeldahl (AOAC, 1990).
[d] Ferrari (AOAC, 1990).
[e] Dirks & Scheffer (AOAC, 1990).
[f] AOAC (1990).

2.2. Field trial set-up

2.2.1. Northern Italy

Field trials were carried out at Legnaro (45°21′N, 11°58′E, 12 m a.s.l., NE Italy) in two consecutive years (2006, 2007) in a fertile silty-loam soil with sub-alkaline pH (Table 1), according to a completely randomised block design with three replications ($n = 3$). The large plot size (~400 m^2) allowed agronomic practices to be managed with farm-scale equipment. The soil was ploughed to a depth of 0.3 m in autumn and harrowed in spring, immediately before sowing. In both years, sowing took place in early spring (March) by means of a mechanical seeder (Gaspardo, Italy), commonly adopted for small cereals, and harvest about three months later (late June) (Table 2). Limited amounts of fertilisers, i.e., 30, 40 and 75 $kg\,ha^{-1}$ of N, P and K, respectively, were applied before sowing and incorporated by harrowing. The inter-row distance was 0.23 m and the amount of seeds 18 $kg\,ha^{-1}$, almost double than recommended, because of the low germination rate of crambe seeds (~ 50%). This amount corresponded to ~250 viable seeds m^{-2}, which provided a final plant density of ~75 plants m^{-2}, for all plots. During the crop cycle, there was no need of either weed or pest control. Main phenological stages were recorded, i.e., emergence, flowering and maturity in each growing season. Plots were entirely harvested at maturity with a Lexion 600 combine harvester equipped with a V600 cutting bar (CLAAS KGaA mbH, Westphalia, D). Plot yield (hulled: seed + capsule) was reported as dry weight (DW) on a hectare base, taking into account seed moisture determined after oven-drying of samples at 105 °C.

2.2.2. Southern Italy

Field trials were carried out at Pozzallo (36°44′N, 14°45′E, 10 m a.s.l., SE Italy) in two consecutive growing seasons (2006–07 and 2007–08, hereinafter referred as 2006 and 2007, respectively) in a red soil of calcareous matrix with sub-alkaline pH (Table 1), according to a completely randomised block design ($n = 3$) with single plot size of 4.0 × 3.0 m. The soil was ploughed to a depth of 0.3 m in early summer and harrowed before sowing. In both years, sowing took place in late autumn (end of November) and harvest in late spring (about mid-May) (Table 2). Mineral perphosphate and

Table 2
Dates of main stages and length of the growing cycle (d, days) for crambe var. Mario grown for two consecutive growing seasons in northern (Legnaro) and southern (Pozzallo) Italy. Thermal time (GDD, Growing Degree Days), cumulate rainfall and mean air temperature (mean T) are reported for site and growing season until and after flowering.

Location	Year	Sowing	Emergence	Flowering	Harvest	Cycle (d)	Until flowering			After flowering		
							GDD[a] (°Cd)	Rainfall (mm)	Mean T (°C)	GDD[a] (°Cd)	Rainfall (mm)	Mean T (°C)
Northern Italy	2006	31 Mar	7 Apr	20 May	5 Jul	96	447	86.0	15.2	735	66.8	21.0
	2007	15 Mar	29 Mar	5 May	26 Jun	103	392	192.2	15.4	828	78.2	21.0
	Mean					100	420	139.1	15.3	782	72.5	21.0
Southern Italy	2006	20 Nov	11 Dec	19 Feb	8 May	169	558	278.6	12.9	791	178.8	19.7
	2007	20 Nov	19 Dec	27 Feb	25 May	183	505	250.6	12.1	913	86.6	20.7
	Mean					176	532	264.6	12.5	852	132.7	20.2

[a] Base temperature for calculation 5 °C (Meijer and Mathijssen, 1996).

potassium sulfate were incorporated by harrowing, by supplying 17.5 and 62.3 kg ha^{-1} of P and K, respectively, whereas 80 kg ha^{-1} of nitrogen (as urea) were dress applied before stem elongation. The inter-row distance and the sowing density were the same adopted in the northern Italy location. During the crop cycle, manual weeding was carried out when necessary, whereas there was no need of pest control. The main phenological stages were recorded during the growing cycle as above. Harvest was carried out manually at physiological maturity. Plot yield (hulled: seed + capsule) was reported as dry weight (DW) on a hectare base, taking into account seed moisture determined after oven-drying of samples at 105 °C.

2.3. Meteorological conditions

Main meteorological data, including air temperature (minimum and maximum) and precipitation, were collected by weather stations located nearby the two experimental locations, and compared with historical averages for each site (Figs. 1 and 2). Growing degree days (GDD) were calculated as follows:

$$GDD = \Sigma(T_{max} + T_{min})/2 - T_{base}$$

where T_{max} and T_{min} are daily maximum and minimum air temperature, respectively, and T_{base} is the base temperature of which a value of 5 °C was adopted (Meijer and Mathijssen, 1996). For each location and growing season, GDD were calculated from emergence to flowering and from flowering till maturity (Table 2). Thermal Use Efficiency (THUE) was calculated for each location as quantity of hulled seed (Kg DM) or oil produced (Kg DM), on hectare base, for each accumulated GDD.

2.4. Seed characterization and analyses

Thousand seed weight (TSW) was calculated as average of three sub-sample measurements for each harvested plot (n = 3), in both locations. The number of seeds produced per unit of soil surface was calculated as the ratio between seed yield and seed weight.

Seed oil content was determined by means of Soxtec-Tecator equipment (FOSS Analytical, 15 Höganäs, Sweden) on 2 g of milled dehulled seeds, with diethyl ether as solvent. Total Fatty Acid Methyl Esters (FAMEs) were analysed only for the 2006 trial set in northern Italy (Legnaro), with three replications (n = 3). After extraction, the oil was prepared according to ISO 5509 (Bondioli and Della Bella, 2002) and analysed in a GC × GC gas chromatograph (Agilent 7980, Milan, I) equipped with an automatic liquid sampler (Agilent 7693, Milan, I). FAMEs were separated on a Supelco SP 2560 capillary column (75 m long, 0.18 mm inner diameter, 0.25 μm film, 0.25 mL min^{-1} flux), and thereafter by a flow modulator (Agilent G35486A, modulation time 2.90 s, sampling time 2.77 s) on a Supelco HP-5 ms (3.8 m long, 0.25 mm inner diameter, 0.25 μm film, 22 mL min^{-1} flux) with hydrogen as carrier. Injection split was set at 160:1 and temperature at 270 °C. The oven temperature program was: initially 45 °C for 2 min, then increased at a rate of 50 °C min^{-1} to 170 °C; this temperature was kept for 25 min, then increased at 2 °C min^{-1} to 240 °C, and lastly held for 16 min. The FID flame ionization detector was set at 250 °C.

2.5. Root investigations

Only at Legnaro (North Italy), in the 2007 growing season, the root profiles of the three crambe varieties were studied with the

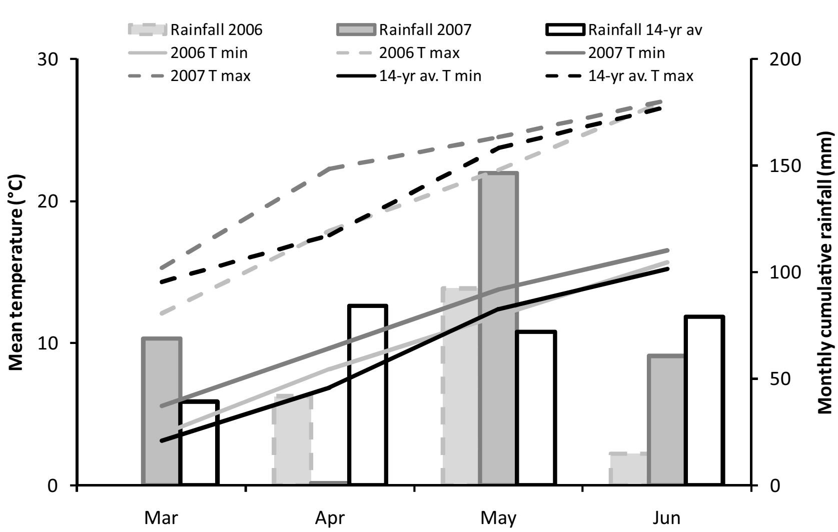

Fig. 1. Monthly cumulate rainfall (histograms, mm) and average minimum (continuous lines, °C) and maximum (dashed lines, °C) temperatures during crambe growing cycle in north Italy (Legnaro) in two growing seasons in comparison with historical 14-year mean (1992–2005).

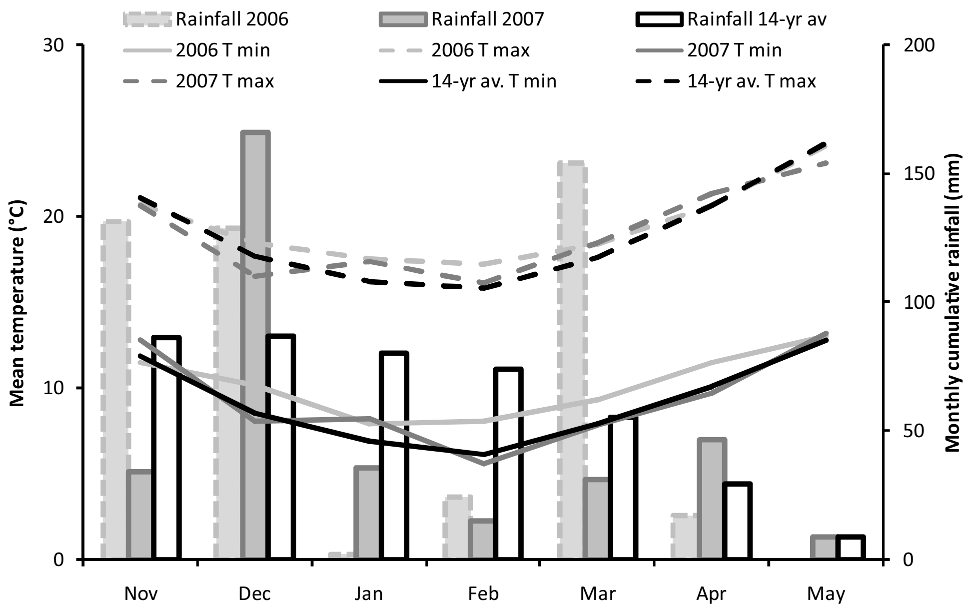

Fig. 2. Monthly cumulate rainfall (histograms, mm) and average minimum (continuous lines, °C) and maximum (dashed lines, °C) temperatures during crambe growing cycle in southern Italy (Pozzallo) in two growing seasons, in comparison with historical 14-year mean (1992–2005).

core sampling method at full flowering (23 May). The root system was investigated to a depth of 1 m in two cores per plot, thus giving 6 replicates per variety. The soil cores were split into 0.1-m sub-samples, which were frozen at −18 °C until washing. Roots were separated from soil particles by a hydraulic sieving-centrifugation device on a 500-μm mesh, allowing any coarse sand to be removed by flotation. Roots were stored in ethanol solution (15% v/v) at 4 °C, and subsequently acquired by digital scanning (Expression 11000XL, EPSON, Milan, I) as 1-bit 400 DPI TIFF format images. Images were processed by KS 300 Rel. 3.0 software (Karl Zeiss, Munchen, D). In image analysis, a minimum area of 20 pixels was set to remove background noise, and an EI (Elongation Index) >50 was chosen to identify roots vs. any extraneous circular objects (e.g., organic debris, weed seeds, etc.). EI was calculated as following:

$$\mathrm{EI} = \mathrm{perimeter}^2/\mathrm{area}$$

Root length was determined by the *FbL* (fiberlength) algorithm, and the mean root diameter as the area-to-length ratio of root objects in a sample (Vamerali et al., 2003).

2.6. Statistical analysis

The results of the all examined parameters were analysed by ANOVA with Statgraphics Centurion XI software (Adalta, Arezzo, I). Comparisons between locations were performed considering year as random effect. Separation of means was set at $P \leq 0.05$ (Newman-Keuls test).

3. Results

3.1. Productive performance

In the southern environment (Pozzallo), crambe showed good adaptation to autumn sowing, whereas in north Italy this option is considered too challenging, in relation to crambe frost sensitivity, thus allowing spring sowing only. Taking into account the relatively short cycle, the seed yield achieved by crambe in both locations and for all tested varieties was appreciable (grand mean: 2.29 Mg DM ha^{-1} of hulled seeds).

In the northern site, in 2006 Mario showed statistically higher seed yield than Galactica and Nebula (Table 3), whereas in the

Table 3
Yield and main productive parameters of three crambe varieties grown in two seasons in northern Italy (Legnaro).

Variety	Year	Seed yield[a] (Mg DM ha^{-1})	Oil content[b] (g DM kg^{-1})	Oil yield (kg ha^{-1})	TSW[c,a] (g DM)	Seed number (m^{-2})
Galactica	2006	3.05b	474a	1013a	5.98b	52197a
Mario		3.26a	444b	1009a	7.33a	44570a
Nebula		3.02b	445b	936b	7.02ab	43591a
Mean		3.11A	4541A	986A	6.78A	46786A
Galactica	2007	2.30a	430b	692a	5.16a	44581a
Mario		2.34a	429b	703a	5.44a	43033a
Nebula		2.21a	449a	697a	5.80a	38115b
Mean		2.28B	4360B	687B	5.46B	41909A
Cv × Yr		ns	*	ns	ns	ns

Small letters: statistical differences among varieties within same parameter. Capital letters: statistical differences between years within same parameter ($P \leq 0.05$, Newman-Keuls test). * = significant for $P \leq 0.05$; ns = not significant.

[a] Hulled seeds.
[b] Dehulled seeds.
[c] Thousand seed weight (TSW).

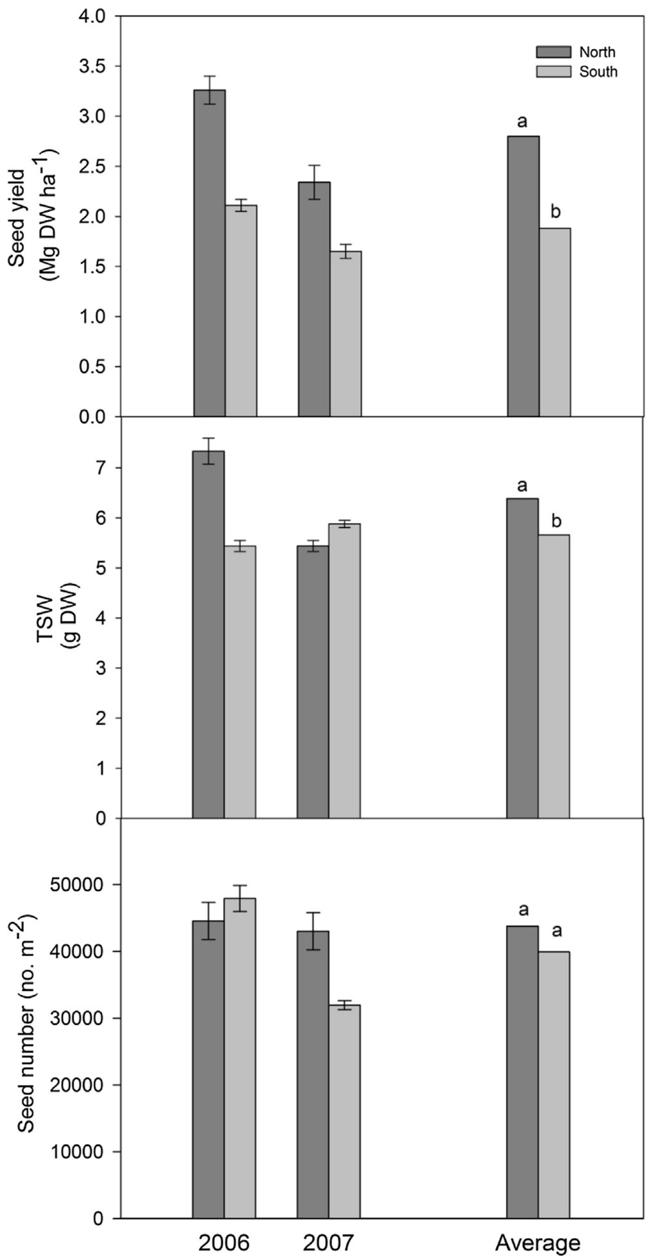

Fig. 3. Seed yield (above), thousand seed weight (TSW, centre), seed production (below) (±*SE*) of crambe var. Mario grown in north (Legnaro) and south Italy (Pozzallo) in two consecutive growing seasons. Letters: significant differences between locations ($P \leq 0.05$, Newman-Keuls test).

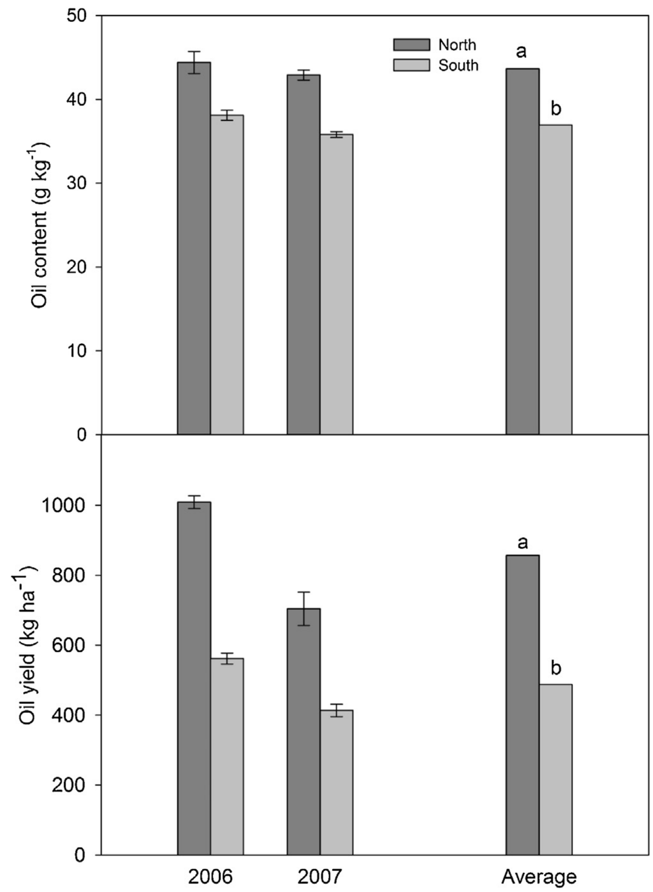

Fig. 4. Oil content (above) and yield (below) (±*SE*) of crambe var. Mario grown in north (Legnaro) and south Italy (Pozzallo) in two consecutive growing seasons. Letters: significant differences between locations ($P \leq 0.05$, Newman-Keuls test).

second year no differences were observed among varieties. Interestingly, environmental conditions demonstrated to be highly impacting on crambe; in particular, in the northern site the second growing season (Table 2) was characterised by a two-fold rainfall until flowering, hampering crop productivity by nearly 30%. The productivity of Mario when grown in southern Italy was also significantly ($P \leq 0.05$) affected by environmental conditions (Fig. 3), it being 22% lower in 2007 vs. 2006 (1.65 vs. 2.11 Mg DM ha^{-1}). Due to spring sowing, in north Italy var. Mario accumulated lower GDD than in the south for both emergence-flowering and flowering-maturity periods (Table 2). Interestingly, productive performances of Mario grown in the two environments demonstrated to be positively correlated with the GDD accumulated until flowering (Table 2). Within each environment, the higher the GDD value accumulated until flowering, the higher the seed yield and oil content (Figs. 3 and 4). Generally, all surveyed productive parameters (i.e., seed yield, TSW, oil yield) showed greater differences (i.e., higher CV) between environments than among varieties grown at the same site.

Considering oil content, it significantly decreased ($P \leq 0.05$) when crambe was grown in southern location (Fig. 4), while the differences among varieties grown in the same conditions, even if significant, were less relevant (Table 3). As a result of the multiplicative effects of seed yield and oil content, differences between locations in oil yield were highly remarkable (867 vs. 488 kg ha^{-1}, north vs. south, respectively) (Fig. 4). Intraspecific variability for TSW was found more relevant (Table 3) than when the same variety (Mario) was grown across different environmental conditions (Fig. 3). In particular, TSW was found quite stable for crambe grown in the southern site, although seeds were significantly smaller than northern site on average. In northern location, Galactica showed smaller seeds (Table 3), with TSW of ~5.6 g (Galactica < Mario < Nebula), negatively correlated with the number of seeds produced per m^2.

As regards oil composition (Table 4), only data for the first growing season (2006) in the northern site are reported, showing slightly higher erucic acid contents (~53%) and significantly lower ($P \leq 0.05$) saturated fatty acids (SFA) in the variety Galactica. Erucic acid production was significantly affected by the variety main effect ($P \leq 0.05$), with the best performances registered in Galactica and Mario.

When analyzing the thermal use efficiency (THUE) for seed and oil yield, highly significant differences emerged between locations. Variety Mario, when grown in northern Italy, reached 70% (2.34 vs.

Table 4
Oil composition of three crambe varieties grown in northern Italy (Legnaro) during 2006. Percentages of main fatty acids and classes, and erucic acid yield.

Variety	Oleic acid	Linoleic acid	Linolenic acid	Eicosenoic acid	Erucic acid	SFA[a]	MUFA[b]	PUFA[c]	Erucic acid yield (kg ha^{-1})
Galactica	16.88b	9.18a	4.37b	3.59a	53.04a	7.82b	75.98ab	13.56b	537a
Mario	16.16c	9.14a	5.00a	3.23a	52.40a	8.30a	74.71b	14.14a	529a
Nebula	17.48a	9.01a	3.92b	4.21a	51.84a	8.40a	76.17a	12.92c	486b
Mean	16.84	9.11	4.43	3.68	52.43	8.17	75.62	13.54	517

Letters: significantly different values among varieties ($P \leq 0.05$, Newman-Keuls test).
[a] SFA: saturated fatty acids.
[b] MUFA: monounsaturated fatty acids.
[c] PUFA: polyunsaturated fatty acids.

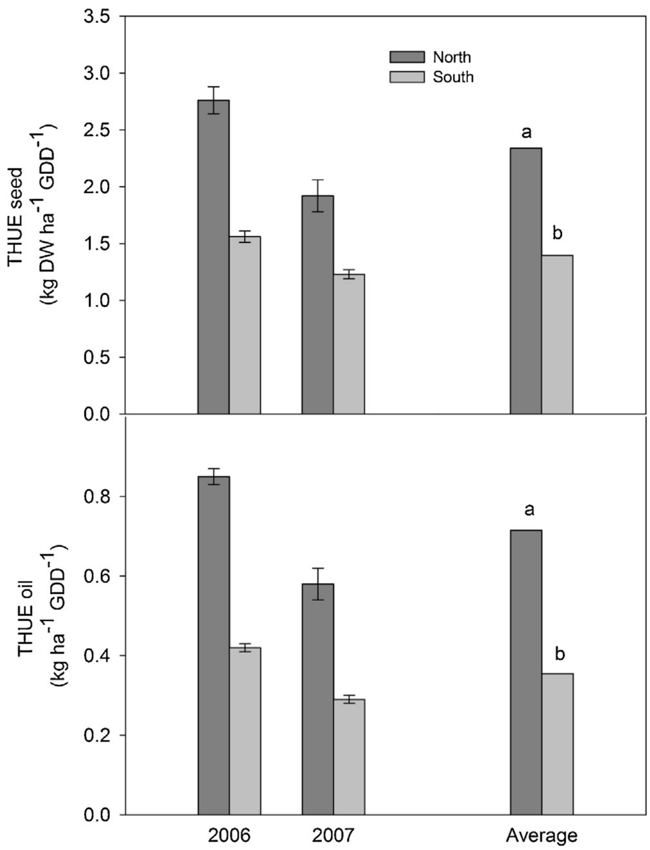

Fig. 5. Thermal energy use efficiency (±*SE*) for seed (above) and oil production (below) in crambe var. Mario grown in north (Legnaro) and south Italy (Pozzallo) in two consecutive growing seasons. Letters: significant differences between locations ($P \leq 0.05$, Newman-Keuls test).

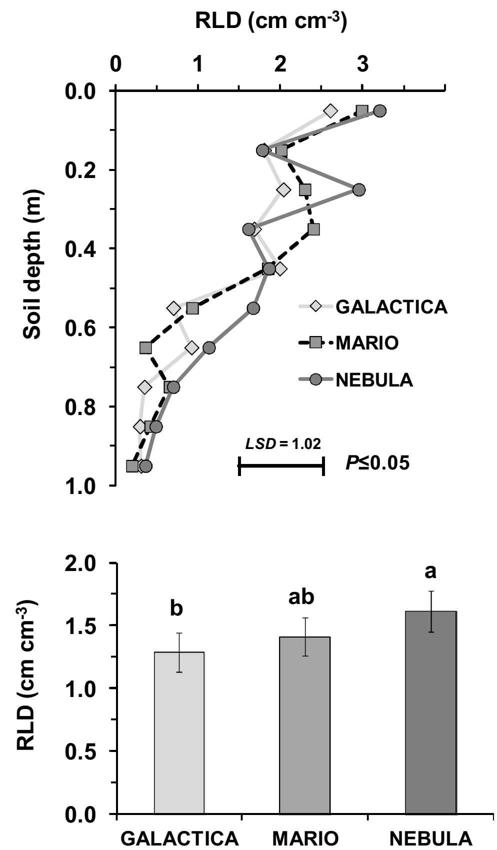

Fig. 6. Root length density (RLD) patterns (above) and average (±*SE*, below) in 1 m soil depth of three crambe varieties in north Italy (Legnaro, 2007). Horizontal bar: *LSD* for 'variety × depth interval' interaction. Letters: significant differences among varieties ($P \leq 0.05$, Newman-Keuls test).

1.68 kg seed GDD^{-1}) and 100% (0.72 vs. 0.35 kg oil GDD^{-1}) higher THUE for seed and oil production, respectively, compared with southern Italy (Fig. 5).

3.2. Root morphology

Information on root morphology and deepening of crambe is currently very limited. Volumetric root length density (RLD) patterns of investigated varieties showed the classical depth-extinction model. In the 1-m deep soil layer, the average RLD was 1.44 cm cm^{-3}, with a median value of 1.48 cm cm^{-3} at 0.5–0.6 m of depth (Fig. 6). In the arable layer (top 0.3 m), RLD of investigated varieties was relatively high, ranging from 2 to 3 cm cm^{-3}, but decreased sharply beneath 0.5 m down to ~0.3 cm cm^{-3} at −1 m. This low density at the maximum investigated depth led to predict a rooting depth of 1.1–1.2 m as maximum. Galactica and Mario had RLD falling below 1 cm cm^{-3} beneath 0.5 m depth, but the value was deeper for Nebula. The average RLD differed among varieties, following the ranking Nebula > Mario > Galactica (1.62 > 1.41 > 1.29 cm cm^{-3}), although only Nebula showed statistically higher values than Galactica (+25%), particularly in the deeper part of the soil profile, and a more stable root pattern (CV 60% vs. 71% and 67% of Mario and Galactica, respectively). A similar trend was determined for root surface area, confirming the aforementioned varietal order (Fig. 7). The total cumulated root surface area approached 20 cm cm^{-2} ground, roughly half the potential for winter wheat in the same location. Due to the close relationship between surface area and length, a stable root diameter is expected in tested varieties (Fig. 8). The general mean (~400 μm) was almost double that of canola and very stable over the whole soil profile, apart from the thinner roots of Nebula in deep soil, which allowed higher specific root length, root length density and area to be achieved.

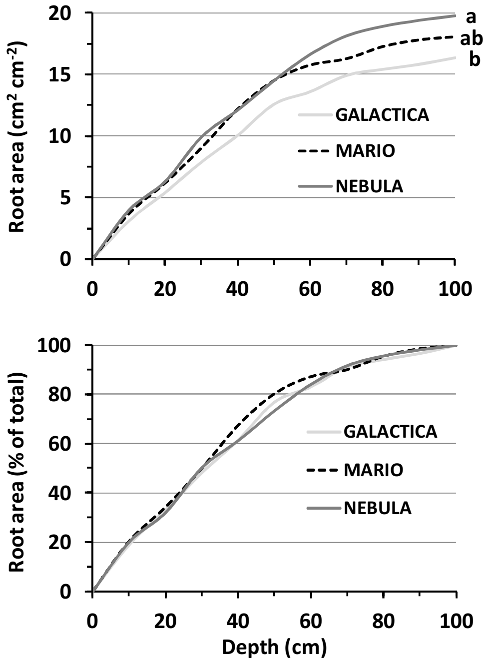

Fig. 7. Cumulate root surface area per unit of ground area (above) and fraction of total (below) over soil profile in three crambe varieties in north Italy (Legnaro, 2007). Letters: significant differences among varieties ($P \leq 0.05$, Newman-Keuls test).

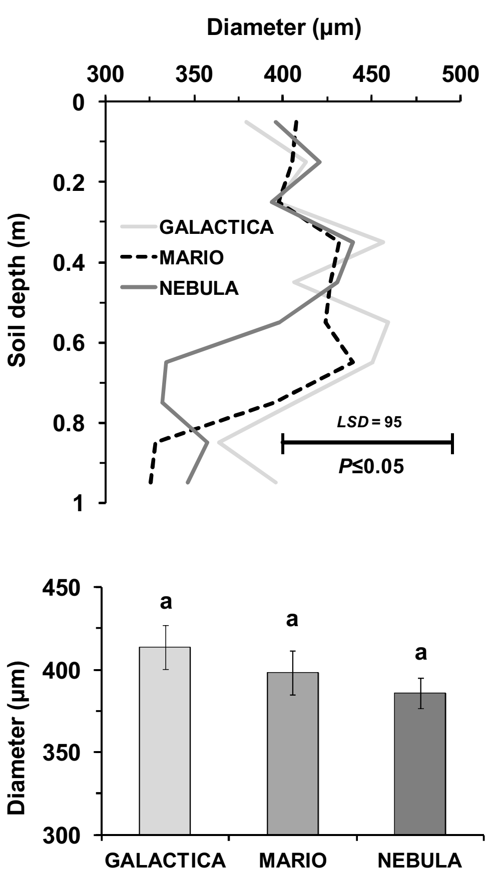

Fig. 8. Patterns of root diameter and average (±*SE*) in 0–1 m soil depth interval of three crambe varieties in north Italy (Legnaro, 2007). Horizontal bar: *LSD* for 'variety × depth interval' interaction. Letters: significant differences among varieties ($P \leq 0.05$, Newman-Keuls test).

4. Discussion

4.1. Productive performance

The growing cycle of crambe (Table 2) among different varieties and contrasting environments appeared quite similar in term of thermal time (420 vs. 532 °Cd until flowering, north vs. south Italy, respectively; and 1202 vs. 1384 °Cd until full maturity, north vs. south, respectively), thus confirming its broad environmental adaptability and low genetic intraspecific variability. Present thermal times are comparable with those of the USDA-ARS variety BelEnzian cultivated in The Netherlands in past experiments by Meijer et al. (1999), although those authors applied a base temperature of 0 °C instead of 5 °C. At the higher latitude of Wageningen (NL), Meijer et al. (1999) found results consistent with those presented here within ranges of 325–438 °Cd from emergence to flower initiation, and 1495–1724 °Cd for seed ripening, depending on sowing time and year. Although crambe is a cool-season crop and high temperatures at the end of the growing cycle may reduce yield, sowing date appears as a key issue in cool winter climates, like that of the northern experimental site, due to the high probability of frost damage. Growth models based on thermal time are generally accurate in predicting phenological phases; uncertainty may arise in suboptimal conditions or under contrasting environmental conditions, as it was found by comparing years or locations. In these trials, crambe showed a good adaptability between locations of the Mediterranean basin, from 36°44′N to 41°21′N (north vs. south Italy), showing appreciable yield potential and in accordance with results by Fontana et al. (1998) and Vollmann and Ruckenbauer (1993). Interestingly, crambe productivity in southern Italy was quite higher than values reported by other authors in similar environments (Laghetti et al., 1995). Intraspecific variability was confirmed to be relatively low (Vollmann and Ruckenbauer, 1993), although substantial variations between years are ascribed to changes in environmental/climatic conditions. In 2007 growing season, in both locations, seed yield, oil content and TSW of the variety Mario were significantly lower than in 2006 (Figs. 3 and 4), probably due to adverse rainfall patterns, confirming the great influence of the environment on crambe productivity (Meijer et al., 1999). In addition, in both sites the reduced thermal time to flowering in 2007 compared with 2006, may have introduced a further source of variability, which contributed to limit yields as a direct consequence of retarded maximisation of photosynthetic active radiation (PAR) interception, as demonstrated by Meijer et al. (1999). These authors emphasized the great importance of early radiation interception (e.g., rosette stage), as after flowering senescence strongly reduces the role of leaves (only 10% of total interception in the last weeks of seed filling). On the other hand, crambe pod area would be too small, even when compared with rapeseed, to replace the role of leaves, it accounting for 30% of light interception together with 50% of stems, that unfortunately are photosynthetically less active structures (Meijer et al., 1999). In crambe, a maximum LAI of 5–6 is commonly estimated to be reached in 50 days after emergence (Meijer et al., 1999), and rapidly decreases to 2 within the following 30 days, while pods (single-seeded) can reach a maximum area of only 0.4 m^2 m^{-2} ground at harvest. In this crop, the source of photosynthates does seem to be the major constraint to high productivity, mainly sustained by early-forming apical branches and pods rather than basal ones.

Out of surveyed productive parameters, seed yield varied most across the two locations (CV 27%) compared with thousand seed weight (16%), number of seeds per m^2 (17%) and oil content, which only slightly changed (9%). When compared within the same environment the variety Mario, improved for the Mediterranean environment, confirmed its generally higher yield potential. However, it was also the most sensitive to climatic variations across years (−28% vs. −26% of Nebula vs. −24% of Galactica: 2007 vs. 2006, north Italy). Seed oil content was also sensitive to climatic conditions, it being lower in south than in north Italy (∼370 vs. ∼430 g kg^{-1}), a response probably related to rapid temperature increase and reduced precipitation typical of early spring in southern Italy (Sicily). By contrast, within the same location the oil content was very stable across varieties and years (CV 4%), confirming once again the low intraspecific genetic variability (Lara-Fioreze et al., 2013).

The lower seed weight of var. Mario when grown in south vs. north Italy was presumably a result of increased temperatures and drought occurring during seed filling stage. Seed production (seeds per m^2) revealed the great potential of crambe to produce seeds across different environments and genotypes. In particular, Mario showed wide adaptability, being able to compensate the lower yield in the southern site with lighter seeds, thus leading to comparable seed production in the two locations (Fig. 3).

As regards oil composition, the erucic acid content (average: ∼52%) was lower than that expected for crambe in similar latitudes (Bondioli et al., 1998; Lazzeri et al., 1994) or in sites further north (Meijer et al., 1999; Vollmann and Ruckenbauer, 1993), where a rate of up to 56% is commonly achieved. Nonetheless, it is worth to note that the erucic acid content of crambe was higher than in HEAR varieties (∼49%) cultivated in the same location under similar low-input management (Zanetti et al., 2009a). In view of the industrial destination of the oil, positive characteristics were also found in Nebula, which is expected to have good oil stability to oxidation thanks to its lower content of polyunsaturated fatty acids (PUFA) (Bondioli, 2003; Erhan et al., 2006). Low temperatures during seed filling are generally expected to increase both PUFA and erucic acid, with a consequent decrease in oleic acid in many high-erucic *Brassicaceae* (e.g., Canvin, 1965; Yaniv et al., 1995): this was presumably an unlikely occurrence in the present environmental conditions, due to the rapid temperature increase after flowering. Trying to compare the adaptability of crambe to differing environments in term of THUE, for both seed and oil productions (Fig. 5), assuming quite stable thermal time across growing seasons and locations (Table 2), the significant variations for THUE are likely to be related to other limiting environmental factors conditioning productivity, than simple degree day accumulation (e.g., rainfall uneven distribution, extreme high temperatures, etc.), that normally occur in southern Mediterranean environment. At this scope further studies including trials, under variegate environmental conditions, are probably necessary to identify the most important factors limiting productivity. Some authors (Adamsen and Coffelt, 2005) identified sowing date as one of the key issues to increase crambe productivity, although it should be decided in relation to total rainfall during crop cycle and its distribution. In the Mediterranean basin, marked uneven rainfall distribution among seasons is generally expected, especially in southern Italy, with precipitations mainly concentrated in autumn/early springtime. If an anticipation of sowing date would presumably lead to safely escape spring/summer drought, in northern Italy this would be linked to low temperatures and consequent slow development, otherwise in southern Italy a further anticipation of sowing to early-autumn would not be feasible since rainfall in that period might not assure a satisfactory establishment of the crop.

4.2. Root morphology

There is very little information in the literature about the root architecture and morphology of crambe, but the reported data seem consistent with those of Merrill et al. (2002, 2005) retrieved from non-destructive minirhizotron video-camera observations under untilled soil. In a 3-year trial, these authors found a maximum depth of 1.18 m and a median one of 0.58 m, both very similar to our findings. Merrill et al. (2002, 2005) demonstrated that crambe had a greater maximum root depth in the drier year, suggesting that this crop can positively respond to adverse conditions. Data on root density and length from minirhizotrons refer to one unit of minirhizotron/soil interface and, although coefficients for their conversion into volumetric root length exist, comparisons with destructive sampling should be carried out with caution. In any case, the presented data are very similar to those of Merrill et al. (2002) in silty soil, but much lower than those reported by Meakin (2007) in drought conditions.

Crambe does appear to have a small root system compared with winter HEAR grown in the same location, year and low-input management (average RLD 0–1 m depth: 1.44 vs. ∼3.60 cm cm^{-3}, crambe vs. HEAR, respectively) (Zanetti et al., 2009b). HEAR and canola have benefited by intense breeding in the last few decades, as demonstrated by the high root efficiency of modern varieties compared with older ones (Vamerali et al., 1999), and improvements are also expected to be achieved in crambe if intense breeding programmes would be applied in the near future. Nowadays, it would be possible to improve crambe root growth and deepening by intensifying agricultural inputs, including N, as observed in HEAR (Zanetti et al., 2009b).

5. Conclusions

After initial investigations in the 1990s, attempts to introduce crambe as a novel oilseed in crop rotations in Europe for the erucic acid industry were abandoned, and still today only a limited number of commercial varieties is marketed. High oil contents and rapid defoliation after flowering greatly hinder high grain yields. However, the particular morphological structure of crambe plant, with stem nodes forming for each single-seeded pod, implies that a positive relationship between total DM accumulation and seed yield does exist, and suggests that genetic and agronomic improvements to the plant biomass would allow higher yields to be achieved. In particular, it could be possible to improve yield stability, which was a major drawback in these trials run under different environments. As crambe has high morphological plasticity, being capable of modulating stem branching as a function of plant density at the present time an increase in sowing density may be a reliable agronomic strategy to reduce both the number of basal shaded branches (often unproductive) and the number of pods per plant, to attenuate the photosynthetic gap in the source-to-sink balance.

Much work must still be done to make crambe more agronomically and economically competitive with HEAR; however its low nutrient requirements, short growing cycle and high thermal use efficiency may make its cultivation in both marginal and fertile soils attractive. Current studies on transgenic lines report substantial improvements in erucic acid contents in the oil (>70%, Li et al., 2012), but there is probably much scope for improving several root features, such as root length density and specific root length and area, which can contribute towards increasing and stabilizing yields in a variety of environmental conditions.

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